Synthetic Organic Chemicals

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The Healing Activity of Organic Compounds Containing the Sulfhydryl Group

A STRIKING example of the application of chemical research in the field of biology is the work being done at the Research Institute of the Lankenau Hospital in Philadelphia by Dr. Hammett, Director of Research, and his associates. A brief account of this investigation is given here since the publications in which the work has been described are not often found in the chemist's library.

The presence of lead salts in solution markedly retards the growth of seedling roots by decreasing the reproduction of the cells by division. An investigation had been started in order to find out if possible the chemical reaction brought about by the lead ions. An analysis of the lead-containing product indicated that it was a combination of the metal with an organic compound containing the –SH group, possibly glutathione. It was further noted that the parts of the root in which the growth by cell division normally occurred most rapidly gave the strongest test with nitroprusside showing a concentration of the sulfhydryl grouping. This led to the suggestion that the sulfur-containing organic compound was closely allied with the growing process.

This was confirmed by showing that a root tip when crushed or scratched with a needle immediately gave a distinct test with the nitroprusside reagent. It was found that extraction with .02 normal hydrochloric acid would remove the stimulating compound from the root tips and that this acid extract could be used to accelerate root growth in other seedlings. Further experiments indicated that these sulfhydryl compounds were always present when through injury or other causes the rapid production of new tissue was required.

A study was immediately begun on the effect of organic compounds containing the –SH group on the growth in length of seedling roots. The roots of small kerneled yellow corn were chosen for the subject of the investigation, and the first sulfur compound tried was thioglycollic acid. This acid was used in very dilute solutions, most of them having a sulfur content of about .01 milligram per hundred cc. In every case the roots immersed in the test solution increased in length more rapidly than the control. When the acid was replaced by its sodium or potassium salt, the same accelerating effect on the growth of the roots was noted. The –SH group was shown to be the determining factor, because when ordinary glycollic acid was used in the same concentration only the normal increase in length resulted.

Even the roots which had been retarded by treatment with lead solutions, after washing out the remaining lead salts, began to grow again when im-

mersed in the thioglycollic acid solution. To see the effect of a more complicated sulfur compound, the amino acid cysteine was used. This, too, stimulated growth more rapidly than in the control experiment where the non-sulfur-containing parallel compound alanine was used. Cystine, the oxidation product of cysteine, containing the –SS– group also showed similar action. Microscopic cell counts demonstrated that the elongation of the roots was not due to lengthening of the original cells, but to their actual subdivision.

The next step was to study the effect of these compounds on a simple form of animal life, for which paramecium was chosen. This required extreme care in the experimental work on account of the sensitivity of these minute organisms. Much more dilute solutions had to be used since thioglycollic acid, even in a concentration of 10^{-7}, kills the paramecium. By means of skilful technic in growing and counting these tiny organisms, it was found that they, too, reproduced more rapidly in the solutions containing sulfhydryl compounds than in the ones which had the corresponding organic compound minus the –SH group. This showed that in the lower forms of animal life as well as in plants, growth by increase in cell number was stimulated by the sulfhydryl group.

The success with paramecium led to experiments with white rats in order to see if the same principle could be applied to more complex and highly developed species. The rats were anesthetized, and two small patches of skin removed from each. The wounds were covered with gauze and cotton and kept moist with the solutions under investigation. The sulfur compound used this time was thioglucose since it was thought that the glucose structure might also provide a utilizable source of energy for the multiplying cells.

One of the dressings on each animal was saturated every two or three hours with a 1:10,000 (based on the sulfur content) thioglucose solution buffered to a pH of 6.8 with a phosphate mixture. The other dressing was kept moist with a similar solution containing glucose instead of thioglucose. At the end of 48 to 72 hours 80% of the rats showed a distinct acceleration in the healing on the side to which the thioglucose had been applied. There was little bleeding on removal of the gauze, the edges of the wound had contracted more rapidly, diminishing the area, and there was considerable cellular growth over the surface. During the same time, however, the wounds which had been treated with the glucose solutions showed but little evidence of healing. This indicated that the stimulating effect of the sulfhydryl group was a fundamental biological phenomenon.

Dr. Reimann, Executive Director of the institute, had followed this work very closely and, encouraged by the successful results in promoting the healing of the wounds in the rats, tried using the extremely dilute thioglucose solution on several patients. One very interesting case was that of a man 76 years old who had suffered from a varicose ulcer on one of his legs for over ten years. In spite of treatment by standard methods, healing had refused to take place. A section of the ulcer was treated with thioglucose solution and within 24 hours it had shown distinct signs of improvement; the surface had begun to granulate and cells had grown out from the edge of the healthy skin. Several other cases treated in a similar manner showed the same improvement, although it was found necessary to discontinue the application after 24 to 48 hours, because the thioglucose apparently stimulated the growth of bacteria as well as normal cells.

To avoid this difficulty, the thioglucose was replaced by a sulfhydryl compound which would tend to inhibit bac-

terial growth, this resulting in marked improvement. The solution used this time was .01 gram of Eastman Thiocresol in 5 cc. of alcohol diluted with 100 cc. of distilled water, giving a concentration of 1:10,000 of the active material. This solution was applied to several stubborn cases of bed sores or ulcers with the result that they immediately began to heal. The application of the solution to the gauze was kept up for 48 hours, then the dressing was replaced for another 48 hours with cotton moistened with a physiologic solution of sodium chloride. These two applications were repeated alternately, resulting in actual spurts in healing during the periods when the thiocresol was used.

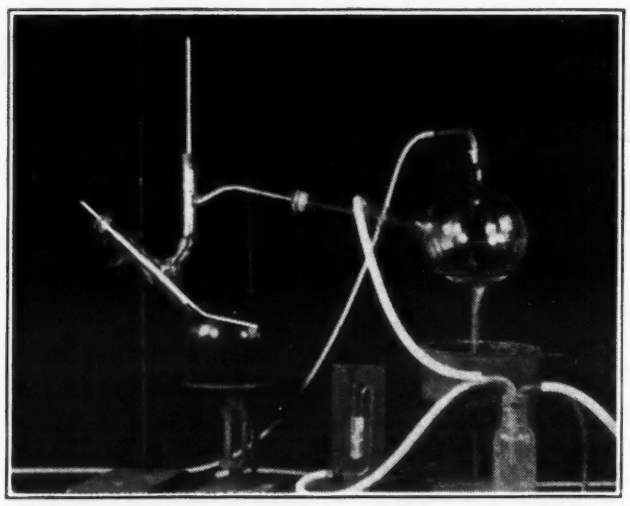
Granulation in the center of the wound grew so rapidly that in several cases it was necessary to remove some of it with silver nitrate in order that the growth at the edges might catch up. There was also a marked decrease in the presence of bacteria over the cases treated with thioglucose. It was found that the solution had to be made up just before use, since the –SH group oxidized rapidly and unless the oxidation products were prevented or removed, healing was slowed up instead of hastened. Further clinical work is in progress in order to determine the most effective sulfhydryl compounds and the best method of using them in the medical field.

The research involved in this problem is a splendid example of the application of chemistry to the biological sciences. Further investigations along these lines may modify the theory outlined above or extend its practical application. At the present time the theory has explained many of the facts that have been noted in cell division and has predicted results in a very practical way, two distinctions that indicate a solid basis for any theory.

"Bumping" Distillations

EVERY organic chemist has had some experience with the distillation of liquids in which boiling does not take place smoothly but occurs in intermittent spurts. Liquids which have been highly purified often have this annoying action since in the distillation process all dissolved air is boiled off and with a perfectly homogeneous liquid, superheating occurs readily.

Small pieces of unglazed tile or capillary tubing are frequently added because they are very helpful. Even with those aids, however, there are times when the entire body of the liquid leaves the bottom of the flask with sufficient velocity to push a hole through the top. Such breakage can be almost entirely avoided by inserting a bent, solid glass rod in the distilling flask with the horizontal portion just above the surface of the liquid. The accompanying photograph shows a vacuum distillation set-up in which this type of baffle rod is used.



When the ascending liquid strikes this simple baffle the velocity is not only retarded, but the surface seems to fold over the rod as an axis so that most of the liquid hits the top of the flask at an angle instead of perpendicularly.

New Eastman Organic Chemicals

The following chemicals have been added to our stock since the publication of the April issue of this bulletin. The ones marked with an asterisk are either made or purified in our own laboratories.

*Adipyl Chloride
Aminopyrene (Practical)
*iso–Amyl Sulfone (Practical)
*Anisoyl Chloride
Antipyrene (Practical)
Brucine (Alkaloid)
Brucine Sulfate
*n-Butyl Adipate
Camphene (Practical)
2.4–Diaminodiphenylamine (Technical)
p.p′–Dichlorodiphenyl (Practical)
*Diethylammonium Diethyldithiocarbamate (Practical)
*3.5–Dinitrobenzoyl Chloride
3–Ethoxy–4–hydroxybenzaldehyde (Practical)
*Ethyl Abietate
Ethyl Abietate (Practical)
Hematein (Stain)
*2.2′.4.4′.6.6′ Hexanitrodiphenyl Sulfide
*Hydrindene Dicarboxylic Acid
1–Mercaptobenzothiazole
*Methyl Adipate
*Methyl Hydrogen Adipate
*β–Phenylpropionyl Chloride
Propenyl Guaiacol (Practical)
*n–Propyl Adipate
*n–Propyl Sulfone
*2.2′.4.4′–Tetranitrodiphenyl Disulfide
Tiglinic Acid (Practical)

Eastman Organic Chemicals as Analytical Reagents

XV. REAGENTS FOR MAGNESIUM

HYDROQUINONE

Yoe, Photometric Chemical Analysis (1928)

In this indirect method, magnesium is separated as magnesium ammonium phosphate. The latter is converted to phosphomolybdate which can be determined colorimetrically according to the Bell and Doisy method by treating with hydroquinone and carbonate-sulfite solution. The magnesium present in the original sample can be calculated from the amount of phosphomolybdate found. This method is used for determining small amounts of magnesium in urine, blood, and tissue extracts.

P–NITROBENZENEAZORESORCINOL

Ruigh, J.A.C.S. *51*, 1456 (1929)

This organic compound in a concentration of 0.5% in 1% sodium hydroxide is a sensitive reagent for the qualitative detection of magnesium. The solution to be tested is made slightly acid with dilute hydrochloric acid and one drop of the reagent added. On making the solution alkaline, a characteristic sky-blue precipitate of the magnesium lake appears. Large amounts of ammonium salts impair the sensitivity so that they should first be "smoked off" in a casserole. Nickel and cobalt give similar lakes but they can be removed easily as sulfides before making the test.

CLAYTON YELLOW

Barnes, C.A. *23*, 1838 (1929)

One half milligram per liter of magnesium in a neutral solution will give an orange color when 10 cc. are treated with a few drops of a 0.1% solution of Clayton Yellow and 0.5 cc. of 4 N sodium hydroxide. With more concentrated solutions the color is brick red. Calcium in quantities less than 300 mg. per liter does not interfere.